Claims

1. A method for the isolation of prostate stem cells comprising the selective enrichment of prostate stem cells which express CD133 antigen.

2. A method according to Claim 1 wherein said stem cells also express high levels of $\alpha_2\beta_1$ integrin.

3. A method according to Claim 1 or 2 wherein said selective enrichment comprises the following steps:

i) providing a cell preparation comprising prostate cells derived from prostate tissue;

ii) providing cell culture conditions which allow the maintenance of said prostate cells in culture and the binding of said prostate cells to a collagen based matrix;

iii) selecting said bound cells wherein said cells express CD133 antigen.

4. A method according to Claim 3 wherein said method includes the additional steps of:

i) culturing cells which express CD133 antigen in culture medium comprising granulocyte macrophage colony stimulating factor (GM-CSF), stem cell factor (SCF) and leukaemia inhibitory factor (LIF); and

ii) passaging the selected cells in (i) in a serum free medium.

5. A method according to any of Claims 1-4 wherein said selected cells express epithelial antigen.

6. A method according to Claim 5 wherein said antigen is human epithelial antigen.

initiate tumour proliferation is determined by varying the number of cells implanted. The self-renewal capacity of the distinct populations is determined by transplanting serially into secondary recipients.

Comparison of gene expression profiles between cancer and normal stem cells

Expression profiles are obtained from stem cells isolated from cancer containing and non-cancer tissue samples. An Affymetrix GeneChip microarray platform is used to assay the absolute gene expression levels for each sample. To accomplish this, total RNA is extracted from purified $\alpha_2\beta_1^{++}$ cells. As the cell yield is low it is necessary to use a linear amplification step to provide sufficient target for hybridisation to the arrays. The Affymetrix small sample labelling protocol has been demonstrated to work well with 100ng (~10^4 cells) but can be used for as little as 1-10ng total RNA. To date we have used this technology (Hu-U133A GeneChips) to profile amplified total RNA extracted from selected cell populations (including $\alpha_2\beta_1^{++}$) derived from our recently isolated prostate cancer lymph node metastasis cell line.

Each sample is derived from a separate individual therefore a substantial degree of variation in gene expression (both between cancer and non-cancer samples and between samples within the same class) will be due to the underling genetic heterogeneity between the individuals. As a result it is necessary to include a number of 'biological' replicates within each class of sample and we typically use 6-10 samples for each (i.e. up to 20 samples in total).

Batch comparison analysis is used to compare each cancer sample experiment to each of the non-cancer samples and subject the comparisons to three different statistical algorithms, based on the Mann-Whitney test, non-parametric Wilcoxon rank test and self-organising map cluster analysis, to detect differential expression. Furthermore, we apply cluster analysis to look for groups of genes which behave differently from the norm.

18 A culture according to Claim 16 or 17 wherein said cells express epithelial antigen.

19. A culture according to Claim 18 wherein said epithelial antigen is human epithelial antigen.

20. A culture according to any of Claims 16-19 wherein cells express CD44 antigen.

21. A culture according to Claim 16 wherein said cells express CD133 antigen, high levels of $\alpha_2\beta_1$ integrin, human epithelial antigen and CD44 antigen.

22. A prostate stem cell preparation obtainable by the method according to any of Claims 1-11 for use as in a vaccine composition.

23. A preparation according to Claim 22 wherein said prostate stem cells are cancerous prostate stem cells.

24. A vaccine composition comprising a prostate stem cell culture according to any of Claims 15-21.

25. A composition according to Claim 24 wherein said composition includes an adjuvant and/or a carrier.

26. A method to immunise an animal comprising administering an effective amount of a prostate stem cell culture according to any of Claims 15-21.

27. A method according to Claim 26 wherein said cell culture comprises cancerous prostate stem cells.

7. A method according to any of Claims 1-6 wherein said selected cells express CD44 antigen.

8. A method according to any of Claims 1-7 wherein said prostate derived tissue comprises cancerous prostate cells.

9. A method according to Claim 8 wherein said cancerous prostate cells are derived from metastatic prostate derived tissue.

10. A method according to Claim 9 wherein said cells are derived from primary and metastic prostate tumours

11. A method according to any of Claims 1-10 wherein said collagen based matrix comprises collagen I.

12. A prostate stem cell obtainable by the method according to any of Claims 1-11.

13. A prostate stem cell according to Claim 12 wherein said cell is a prostate cancer stem cell.

14. A prostate stem cell according to Claim 13 wherein said stem cell is cloned.

15. A cell culture of substantially pure prostate stem cells wherein said cells express CD133 antigen.

16. A culture according to Claim 15 wherein said cloned cells are prostate cancer stem cells.

17. A culture according to Claim 16 wherein said cells express high levels of $\alpha_2\beta_1$ integrin.

iv) detecting a signal which indicates the binding of said nucleic acid to a binding partner on said nucleic acid array.

39. A method according to Claim 38 wherein said method includes the additional steps of:

i) collating the signal(s) generated by the binding of said nucleic acid to said binding partner;

ii) converting the collated signal(s) into a data analysable form; and optionally;

iii) providing an output for the analysed data.

40. A method according to Claim 38 or 39 wherein said preparation comprises cancer prostate stem cells.

41. A method according to any of Claims 38-40 wherein said method includes a comparison of the array signal produced between normal and cancer prostate stem cells.

42. A method according to any of Claims 38-40 wherein said method includes a comparison of the array signal produced between a first cancer prostate stem cell sample and a second, different, cancer prostate stem cell sample.

43. A method for the preparation of a library comprising prostate specific gene expression products comprising the steps:

i) providing a preparation comprising at least one prostate stem cell according to any of Claims 12-14;

ii) extracting nucleic acid from said cell preparation;

iii) preparing a cDNA from ribonucleic acid contained in said extracted nucleic acid; and

iv) ligating cDNA formed in (iii) into a vector.

28. A method according to Claim 26 or 27 wherein said animal is a human.

29. A method according to Claim 26 or 27 wherein said animal is a rodent.

30. A method according to Claim 26 or 27 wherein said animal is a rabbit, goat or sheep.

31. A method according to Claim 26 or 27 wherein said animal is a dog.

32. An antibody obtainable by the method according to any of Claims 26-31.

33. An antibody according to Claim 32 wherein said antibody is a monoclonal antibody or binding fragment thereof.

34. An antibody according to Claim 32 or 33 for use as a pharmaceutical.

35. A pharmaceutical composition comprising an antibody according to Claim 32 or 33.

36. A T-lymphocyte obtainable by the method according to any of Claims 26-31.

37. A T-lymphocyte according to Claim 36 wherein said lymphocyte is a T-helper lymphocyte.

38. A method for the identification of genes that show enhanced expression in prostate stem cells comprising the steps of:

i) providing a preparation comprising at least one prostate stem cell according to any of Claims 12-14;

ii) extracting nucleic acid from said cell preparation;

iii) contacting said extracted nucleic acid with a nucleic acid array; and

50. A method to identify agents capable of inhibiting the motility of cancerous prostatic cells comprising:

i) providing culture conditions and at least one cancerous acinus according to Claim 47 or 48;

ii) adding at least one agent to be tested; and

iii) monitoring the motility of cells comprising the cancerous acinus.

51. A method to identify markers of prostate cell differentiation comprising the steps:

i) providing a preparation comprising prostate stem cells according to any of Claims 12-14; and

ii) determining the expression of at least one gene the expression of which is associated with the differentiation of prostate cells.

52. A method to identify markers of prostate cell transformation

i) providing a preparation comprising prostate stem cells according to any of Claims 12-14; and

ii) determining the expression of at least one gene the expression of which is associated with the transformation of prostate cells.

53. A method according to Claim 52 wherein said gene is an oncogene.

54. A method according to Claim 53 wherein said oncogene is encoded by a nucleic acid molecule comprising a nucleic acid sequence as represented in Figure 3, or a nucleic acid molecule that hybridises to said nucleic acid under stringent hybridisation conditions and which encodes a polypeptide with transcription factor repressor activity.

55. A method according to Claim 54 wherein said nucleic acid encodes a polypeptide as represented by the amino acid sequence shown in Figure 4, or a

44. A method according to Claim 43 wherein said vector is a phage based vector.

45. An *in vitro* method for the formation of prostate-like acini comprising:

i) providing a cell culture vessel comprising:

a) prostate stem cells according to any of Claims 12-14;

b) a cell culture support matrix to which the cells in (a) can attach and proliferate;

c) cell culture medium supplemented with serum, a stromal fraction and a ratio of the hormones oestrogen and dihydrotestosterone, or functional derivatives thereof; and

ii) providing conditions which promote the growth and differentiation of said prostate derived cells in said vessel.

46. A prostate-like acinus obtainable by the method according to Claim 45.

47. A cancerous prostate-like acinus obtainable by the method according to Claim 45.

48. A prostate-like acinus according to Claim 46 or 47 wherein said acinus comprises genetically engineered prostate cells.

49. A method to identify agents capable of inhibiting the proliferation of cancerous prostatic cells comprising:

i) providing culture conditions and at least one cancerous acinus according to Claim 47 or 48;

ii) adding at least one agent to be tested; and

iii) monitoring the anti-proliferative activity of the agent with respect to the cells comprising the cancerous acinus.

62. A method according to Claim 61 wherein said method detects expression of mRNA.

63. A method according to Claim 62 wherein said method is a polymerase chain reaction method.

64. A method according to Claim 62 wherein said method detects a polypeptide encoded by said nucleic acid molecule.

65. A method according to Claim 64 wherein said polypeptide is detected by an antibody specifically reactive with a polypeptide as represented by the amino acid sequence as shown in Figure 4.

66. A non-human animal model for the analysis of the formation of prostate acini comprising the steps of:

i) providing a preparation of prostate stem cells;

ii) transplanting said cells into a non human animal subject; and

iii) monitoring the differentiation and growth of the transplanted cells.

67. A non-human animal model according to Claim 66 wherein said animal is selected from the group consisting of: mouse, rat, guinea pig, dog, non-human primate.

68. A non-human animal model according to Claim 67 wherein said animal is an immune compromised mouse.

69. A non-human animal model according to Claim 68 wherein said mouse is a SCID mouse or an athymic nude mouse.

70. A non-human animal model according to any of Claims 66-69 wherein said cells are transplanted subcutaneously.

variant amino acid sequence that has been modified by addition, deletion or substitution of at least one amino acid residue and has transcription factor repressor activity.

56. The use of a polypeptide as represented by the amino acid sequence shown in Figure 4, or a variant amino acid sequence that has been modified by addition, deletion or substitution of at least one amino acid residue, for the manufacture of a vaccine composition for use in the immunisation of a subject against prostate cancer.

57. The use of a nucleic acid molecule comprising a nucleic acid sequence as shown in Figure 3, or a nucleic acid molecule that hybridises to said nucleic acid under stringent hybridisation conditions, for the manufacture of a vaccine composition for use in the immunisation of a subject against prostate cancer.

58. The use of an antibody specifically reactive with a polypeptide as represented by the amino acid sequence shown in Figure 4, for the manufacture of a medicament for use in the treatment of prostate cancer.

59. Use according to Claim 58 wherein said antibody is a monoclonal antibody, or an active binding fragment thereof.

60. Use according to Claim 59 wherein said antibody fragment is a single chain antibody variable region fragment or a domain antibody.

61. A method of screening a subject for prostate cancer, or a predisposition to prostate cancer, comprising the steps of:

i) providing an isolated sample comprising prostate cells; and

ii) detecting the expression of a nucleic acid molecule comprising a nucleic acid sequence as shown in Figure 3, or a nucleic acid molecule that hybridises to said nucleic acid under stringent hybridisation conditions.

71. A non-human animal model according to any of Claims 66-69 wherein said cells are transplanted orthotopically in or around prostate tissue.

72. An *in vitro* method for the formation of vascularised prostate acini comprising the steps of:

i) providing a cell culture vessel which includes: prostate-like acini formed from prostate stem cells according to the invention that have been formed in a cell culture medium supplemented with serum, a stromal fraction, and a ratio of oestrogen and dihydrotestosterone, or functional derivatives thereof, a cell culture support matrix; and a cell culture medium which supports the growth of said prostate acini; and

ii) addition of activated endothelial cells to said cell culture vessel wherein said endothelial cells proliferate and/or migrate to form blood vessel tubules in or around said acini.

73. An *in vitro* method for the formation of vascularised prostate-like acini comprising:

i) providing a cell culture vessel which includes:

a) prostate stem cells and activated endothelial cells;

b) a cell culture support matrix to which the cells in (a) can attach and proliferate;

c) cell culture medium supplemented with serum, a stromal fraction and a ratio of the hormones oestrogen and dihydrotestosterone, or functional derivatives thereof; and

ii) providing conditions which promote the growth and differentiation of said prostate epithelial cells in said vessel to form prostate acini and the vascularisation of said acini by the formation of blood vessel tubules from said activated endothelial cells.

74. A method according to Claim 72 or 73 wherein said prostate stem cells and endothelial cells are of human origin.